

Lack of pharmacokinetic bioequivalence between generic and branded amoxicillin formulations. A post-marketing clinical study on healthy volunteers

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Generic medicinal products are 'copies' of patented drugs and can be marketed at low cost following patent expiration of the brand-name preparations.
- Although the development of generic medicinal products is regulated by specific guidelines, a number of issues and concerns continue to undermine the confidence of physicians and patients in generic drugs.

WHAT THIS STUDY ADDS

- The present findings open interesting perspectives for the discussion of the quality of generic drugs in the postmarketing setting.
- In particular, our trial shows that postmarketing evaluation of bioequivalence between branded amoxicillin and its generic copies might result in lack of interchangeability.

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AIMS

There are concerns about the quality of generic drugs in the postmarketing setting. The aim was to establish whether two generic formulations of amoxicillin, available on the Italian market, fulfil the criteria for clinical pharmacokinetic bioequivalence vs. the branded drug.

METHODS

Two generic amoxicillin products (generic A and B) were selected among four fast-release tablet formulations available on the Italian market. Twenty-four healthy adult volunteers of either sex participated to a single-dose, randomized, three-treatment, crossover, single-blind bioequivalence study designed to compare generic A and B with branded amoxicillin. Plasma samples were collected at preset times for 24 h after dosing, and assayed for amoxicillin levels by high-performance liquid chromatography.

RESULTS

Ninety percent confidence intervals of AUC ratios were 0.8238, 1.0502 (ratio 0.9302) and 0.8116, 1.1007 (ratio 0.9452) for generic A and B vs. branded amoxicillin, respectively. Ninety percent confidence intervals of C_{max} ratios were 0.7921, 1.0134 (ratio 0.8960) and 0.8246, 1.1199 (ratio 0.9610) for generic A and B vs. branded amoxicillin, respectively. The mean pharmacokinetic profiles showed that the AUC value of branded amoxicillin was 8.5 and 5.4% greater than that estimated for generic A and B, respectively. Few adverse events were recorded; these were not serious and occurred without apparent relationship to any specific amoxicillin formulation.

CONCLUSIONS

These results indicate that one of the two marketed amoxicillin generics analysed in the present study is not bioequivalent to the brand leader product for C_{max} on the basis of single-dose pharmacokinetic assessment.

Introduction

Generic medicinal products are 'copies' of patented drugs and can be marketed at low cost following patent expiration of the brand leader preparation. The main purpose of generic drug development is to reduce the price of marketed drugs, ultimately to lower public health costs. As a consequence of increasing restrictions on the economic resources allocated to public health programmes, many governments strongly support the production and clinical use of generic medicinal products in place of reference brand-name drugs. Accordingly, the regulatory authorities of several countries, including the Food and Drug Administration, the European Agency for the Evaluation of Medicinal Products (EMA) and the World Health Organization (WHO), have issued guidelines illustrating the terms and conditions under which generic drug products can be recognized as therapeutically equivalent to their brand-name counterparts [1–3].

Scant knowledge of the procedures for the registration of generic drugs has led many patients and physicians to presume that a generic product should be identical in all respects to the brand leader drug originally introduced onto the market [4]. By contrast, the chemical composition of generic formulations may differ from their respective brand products. Indeed, the use of different excipients is commonly allowed by international guidelines under specific terms and conditions [1, 3]. As regards the active ingredients, these molecules can be present in generic formulations as different salts or polymorphic species of the leader compound. In particular, the EMA guideline designates as 'pharmaceutical alternative' a medicinal product that contains a different chemical form (i.e. salt, ester, etc.) of the active ingredient present in the brand leader [1].

Bioequivalence studies, consisting of single-dose pharmacokinetic evaluations, are required for the registration of most generic drug formulations. In general, bioequivalence testing is regarded as a useful methodology to perform comparisons among different products containing the same active ingredient. In this respect, bioequivalence studies are also suitable for the clinical development of a new chemical entity as well as in the postmarketing phase of the brand leader [5]. Nevertheless, some authors claim that single-dose bioequivalence studies in healthy volunteers might not predict the actual therapeutic equivalence in patients who receive the drug as repeated dosing regimens. Furthermore, concerns are being raised on the lack of interchangeability between branded and generic drugs in the postmarketing setting. For example, according to Crawford *et al.* [6], switching from branded antiepileptics to generic copies might result in increased risk of therapeutic failure or adverse reactions. Thus, despite efforts by regulatory authorities to care for patient health when granting applications for generic drug registration, physicians and patients might have prejudices against generic drug substitution.

In some instances, guidelines support the use of *in vitro* dissolution studies to test the bioequivalence of generic drugs formulated as oral fast-release tablets, without any need for clinical pharmacokinetic or pharmacodynamic investigations [3, 7], and WHO has published a list of drugs for which biowaiver applications could be submitted [8]. However, the use of *in vitro* dissolution tests as surrogates of *in vivo* studies applies only to class I drugs (i.e. high permeability, high solubility), and additional restrictions are indicated in notes issued by EMA [9]. In accordance with EMA recommendations, in most cases the Italian regulatory authority requires the demonstration of *in vivo* bioequivalence for the registration of generic drugs and allows biowaiving of *in vivo* testing only in a restricted number of circumstances [1, 9].

Antibacterial drugs include several pharmacological classes, the therapeutic activity of which depends significantly on pharmacokinetic and pharmacodynamic parameters, such as C_{max} (highest drug concentration achieved in plasma), AUC (area under the drug plasma concentration–time curve) and the time during which plasma concentrations are higher than minimum concentration inhibiting bacterial growth (MIC) [10]. For generic antibiotics, differences in pharmaceutical properties might result in changes of their pharmacokinetic profiles, with consequent alteration of pharmacokinetic/pharmacodynamic relationships, leading ultimately to variations in their clinical efficacy with respect to the brand-name counterparts. Thus, it appears of interest to evaluate the pharmacokinetic bioequivalence of generic antibiotics in the postmarketing setting, to verify that patients are provided with generic products of adequate quality.

The β -lactam amoxicillin is usually employed for short-term antibacterial treatments but, in some instances, it can be administered orally on a long-term basis [11], and in Italy it is marketed as both branded and a number of generic copies. Therefore, based on the above considerations, the present study was undertaken to establish whether two generic formulations of amoxicillin, available on the Italian market, fulfil the criteria for clinical pharmacokinetic bioequivalence vs. their reference brand product. Care was also taken to compare the pharmacokinetic patterns of the two generic amoxicillin preparations.

Methods

Volunteers

Twenty-four healthy adult volunteers of either sex were invited to participate to a single-dose, randomized, three-period, three-treatment, crossover, single-blind pharmacokinetic bioequivalence study. At the time of enrolment, the volunteers were informed of the purpose, duration and risks of the study, and they were requested to sign a written informed consent. They were also informed about the

Table 1

Chemical composition of branded and generic amoxicillin formulations

Amoxicillin formulations	Active ingredient	Excipients
Branded	Amoxicillin tetrahydrate	Natrium carboxymethyl amide Colloidal silica Magnesium stearate Microcrystalline cellulose
Generic A	Amoxicillin tetrahydrate	Natrium carboxymethyl amide Colloidal anhydrous silica Magnesium stearate Mais amide Povidone
Generic B	Amoxicillin tetrahydrate	Natrium croscamellose Magnesium stearate Microcrystalline cellulose
Generic C	Amoxicillin tetrahydrate	Natrium carboxymethyl amide Magnesium stearate Microcrystalline cellulose Talc Precipitated silica
Generic D	Amoxicillin tetrahydrate	Natrium carboxymethyl amide Magnesium stearate Microcrystalline cellulose

possibility of withdrawing from the study at any time. They were not allowed to consume alcohol or beverages and foods containing caffeine from 48 h prior to drug administration until the end of the study. Women were screened for β -human chorionic gonadotropin in urine to rule out ongoing pregnancies, and they were then requested to use nonpharmacological contraceptive devices throughout the study period. The volunteers were also instructed to abstain from taking any medication during 4 weeks before and throughout the whole course of the study, and they underwent careful clinical examinations both before and after participation in the study. The evaluations and tests performed included: medical history, physical examination, height, weight, body mass index, vital signs (heart rate, systolic and diastolic blood pressure, body temperature), renal and liver function tests and electrocardiogram. The volunteers were requested to report any abnormality occurring throughout and after the study. The results of clinical evaluations were documented in individual case report forms. The study protocol was approved by the Ethics Committee of Pisa University Hospital.

In vitro selection test

Two generic amoxicillin formulations, to be employed in the clinical trial, were chosen among four products available on the Italian market (Table 1) by means of an *in vitro* selection test. Since the *in vitro* test was aimed at performing a preliminary screening, and not for regulatory purposes, the experimental procedures were modified and simplified with respect to the reference method suggested by US Pharmacopoeia [12]. Briefly, for the branded and each generic amoxicillin formulation (designated as A, B, C

and D), 1000-mg tablets were allowed to dissolve completely in a volume of 350 ml of phosphate-buffered saline, pH 2.0, at 37°C, under continuous stirring, and 200- μ l samples of medium were collected at different time points (3, 5, 10, 15, 25 and 40 min). The *in vitro* selection test was repeated in triplicate. The concentration profiles of generic tablets were compared with that of the brand leader by calculating the amount of amoxicillin dissolved into the medium over 40 min, expressed as percentage areas under the time–concentration curves.

Study design

Healthy volunteers were randomized in three groups of eight subjects, and each group received the three drug treatments at three different times, with an intervening 1-week wash-out period. A simplified three-sequence design was applied to the present trial, since amoxicillin has a short plasma half-life, it has not been reported to exert inducing/inhibiting metabolic activity [13] and a wash-out period of 7 days was regarded as sufficient to avoid a carry-over effect. Thus, each volunteer received branded or generic amoxicillin, as 1000-mg tablets at different days, in accordance with the crossover design. The tablets were administered with 250 ml of water at 08.00 h after overnight fasting. Lunch and dinner were served 4 and 10 h after dosing, respectively. Venous blood samples of 5 ml were collected, via an indwelling cannula placed on the forearm, into Vacutainer™ tubes (containing sodium heparin) at preset time intervals of 0 (predose), 0.5, 1, 2, 4, 6, 8, 12 and 24 h after dosing. The blood samples were centrifuged at 900 g for 15 min, plasma samples were transferred to Vacutainer™ tubes (no additive) and stored at –80°C until subsequent analysis.

Tolerability evaluation

Volunteers were asked about the occurrence of any adverse event after their admission to the clinical unit, before administration of the test drugs, and approximately every 4 h thereafter until discharge. Clinical evaluations, performed at screening, were repeated within 15 days from the end of the study to detect putative adverse events. Radial pulse and blood pressure were monitored as vital signs.

Pharmacokinetic evaluation

Noncompartmental analysis to calculate pharmacokinetic parameters was performed with WinNonlin version 4.0 (Pharsight, Mountain View, CA, USA). The actual times of sample collection were used for pharmacokinetic analyses of branded and generic amoxicillin formulations. AUC from time 0 to infinity ($AUC_{0-\infty}$) was estimated by the linear trapezoidal method, calculated as the sum of AUC from time 0 to 24 h plus the ratio of the last measurable plasma concentration to the elimination rate constant. C_{max} and T_{max} (the time to achieve C_{max}) were obtained from direct visual inspection of plasma concentration vs. time curves. Moreover, to assess the appropriateness of the blood sampling schedule, T_{max} was also estimated by pharmacokinetic analysis after interpolation of raw data.

Amoxicillin assay

Amoxicillin concentration in plasma and buffer samples was measured by high-performance liquid chromatography (HPLC) in accordance with the method of Du *et al.* [14]. Briefly, a stock solution of amoxicillin (1000 mg l^{-1}) was prepared in deionized water, and further diluted in pooled normal human plasma to obtain calibration and quality control (QC) samples. In particular, calibration standards were spiked with amoxicillin stock solution to give final concentrations of 0.3125, 1.25, 2.5, 5, 10 and 40 mg l^{-1} . Aliquots of these standard solutions were stored at -80°C until analysis. Three QC samples were prepared by using the same procedure at concentrations of 0.3125, 2.5 and 5 mg l^{-1} in human serum and 0.3125, 2.5 and 5 mg l^{-1} in saline, respectively. The stock solution of cefadroxil [internal standard (IS)] was prepared in deionized water at a concentration of 1000 mg l^{-1} . Aliquots of this solution were stored at -80°C until use. When determining unknown samples, the QC measurements were randomly distributed among the unknown samples to be extracted and injected. Each sample was assayed in duplicate. The accuracy of all QC samples was examined to confirm the assay validity.

Extraction procedure

Two-hundred microlitres of unknown standard and QC plasma samples were added with $20 \mu\text{l}$ of IS solution. Plasma proteins were removed by precipitation, adding $500 \mu\text{l}$ of acetonitrile, shaking the samples vigorously for

5 min and centrifuging at $2250 g$ for 5 min. The supernatant was transferred to a clean tube and 2 ml of dichloromethane was added. After shaking slowly for 5 min and centrifuging at $2250 g$ for 5 min, $100 \mu\text{l}$ of the top layer was collected and $20 \mu\text{l}$ injected into the HPLC apparatus. A Waters 2695 Alliance HPLC system, equipped with a 2764 UV detector, was used for amoxicillin assay. In particular, the autosampler temperature was set at 10°C , while the detection wavelength was 210 nm. A Phenomenex C_{18} column ($4.6 \times 250 \text{ mm}$, $10 \mu\text{m}$; Phenomenex, Torrance, CA, USA) was used as analytical column, with a $\mu\text{Bondpak } C_{18}$ Guard-pack precolumn (Waters, Milford, MA, USA) maintained at room temperature. The mobile phase consisted of phosphate buffer (0.023 mol l^{-1} , pH 3.0) containing 4 mmol l^{-1} 1-octanesulphonic acid sodium and acetonitrile (87 : 13, v/v), and the flow rate was set at 1 ml min^{-1} . Chromatographic data were collected by using Empower version 2 software (Waters).

Validation of HPLC assay

The peak height ratio (PHR) of amoxicillin to cefadroxil was used for all calculations. Calibration curves, with six concentration points, were constructed by plotting PHR vs. spiked concentrations. The weighed least square linear regression (weighing factor: $1/\text{concentration}$) was selected, since calibration curves spanned a range of nearly 100. The calibration curves were used to calculate amoxicillin concentration both in QC and unknown samples. Recovery of amoxicillin and IS from human plasma and saline was calculated by comparing peak heights of amoxicillin and IS of extracted QC samples with those of aqueous solution at the same concentration. The precision of the method was determined as intraday and interday variability of low, medium and high concentration QC samples. Accuracy was evaluated by the relative bias of calculated concentrations of QC samples compared with their theoretical values.

Statistical analysis

The parametric general linear model for statistical analysis included factors accounting for sequence effect, subjects included in nested sequences, period and treatment. Considering the number of subjects recommended by current guidelines (usually 12 for the 2×2 design) [1, 3], we assumed that the enrolment of 24 volunteers ensured a sufficient power associated with the test. Accordingly, coefficient of variation values yielded from analysis of variance (ANOVA) were below the recommended upper limit of 30%, and the power associated with the two Schuirman *t*-tests, used to compare the generic products with branded amoxicillin, was $>90\%$. Pharmacokinetic bioequivalence comparison was carried out by the statistical software EquivTest/PK (Statistical Solutions Unit7B, Farmer's Cross, Ireland) in accordance with EMEA guideline [1]. C_{max} and AUC values were log-natural transformed and used to calculate the ratios of each test drug over the respective

reference product. The 90% confidence interval (CI) of ratios was calculated to assess the bioequivalence of each generic formulation vs. the branded product as well as between the two generic formulations.

Results

Characteristics of healthy volunteers

Twenty-four subjects, who met the selection criteria, consented to participate in the study and completed the experimental procedures. The overall characteristics of healthy volunteers are reported in Table 2. ANOVA showed that there were no differences between groups of volunteers in terms of age, height, weight or body mass index ($P > 0.05$).

Safety

No serious or significant adverse events occurred throughout the study. One case of photosensitization and dermatitis (moderate) was recorded after the third treatment. This adverse event resolved within 2 weeks and it was judged as related to study drugs but not ascribable to any specific formulation of amoxicillin. Fifteen volunteers experienced mild headache after dosing. However, these adverse events were not considered as related to study drugs. One volunteer reported mild nausea on dosing days, probably as a consequence of amoxicillin intake, without apparent relationship to any specific formulation. One subject developed mild diarrhoea that was probably related to amoxicillin. Mild periocular oedema and fever were also observed during the study and these events were not regarded as drug related.

Validation of HPLC method

The relationship between concentration and peak area ratio was found to be linear within the range of 0.3125–40 $\mu\text{g ml}^{-1}$ ($r^2 = 1.00$ with quantification and detection limits of 0.3125 and 0.1 mg l^{-1} , respectively). The precision, expressed as the percentage of coefficient of variation, was found to be $<15\%$ in all assayed concentrations. The intraday precision of the method was $<15\%$ and the accuracy value (percentage of error) was $<15\%$. Interday precision values were found to be $<15\%$. The recovery ranged from

Table 2

Subject demographics

	Age (years)	Weight (kg)	Height (m)	BMI
Group 1 (n = 8)	30.3 \pm 3.5	69.6 \pm 15.5	1.73 \pm 0.12	22.7 \pm 1.8
Group 2 (n = 8)	28.3 \pm 6.3	63.0 \pm 12.2	1.69 \pm 0.09	21.8 \pm 1.9
Group 3 (n = 8)	28.5 \pm 4.0	62.8 \pm 11.0	1.67 \pm 0.10	22.2 \pm 1.6
	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$

Data are given as mean \pm standard deviation. BMI, body mass index.

91.4 to 105% for amoxicillin concentrations of 0.3125–40 mg l^{-1} . The stability test showed that amoxicillin was stable in plasma for 6 weeks when stored at -80°C .

In vitro selection test

The *in vitro* test showed that, among the four generic formulations selected for the present study, generic A and B displayed the greatest differences in their concentration–time profiles, because of a slow increase in amoxicillin buffer concentration over time. Analysis of *in vitro* profiles revealed that the dissolved amounts of generic A and B were 81.6 and 43.1% with respect to branded amoxicillin, whereas those of generic C and D were 85.4 and 90.2%, respectively. Thus, generic A and B were chosen as test drugs for the clinical trial of pharmacokinetic bioequivalence vs. branded amoxicillin.

Pharmacokinetic evaluations

The mean plasma profiles of all 24 subjects, exposed to brand or generic amoxicillin formulations, are shown in Figure 1. The respective values of estimated pharmacokinetic parameters are reported in Table 3, as both arithmetic and geometric means. Of note, the T_{max} values obtained after pharmacokinetic analysis by interpolation of individual plasma profiles of branded, generic A and B amoxicillin (1.90 ± 0.97 , 1.78 ± 0.80 and 1.81 ± 0.82 h, respectively) did not differ appreciably from those estimated by visual inspection (Table 3), supporting the appropriateness of the blood sampling time schedule. Branded amoxicillin showed the highest C_{max} and AUC values. The AUC value of branded amoxicillin was 8.5 and

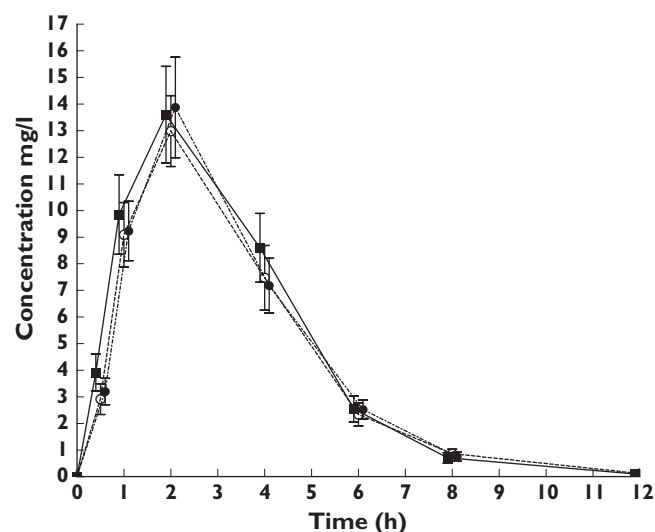


Figure 1

Mean amoxicillin concentration–time profiles in plasma following the administration of branded or generic amoxicillin formulations to healthy volunteers. Each point represents the geometric mean \pm standard deviation (vertical lines) obtained from 24 subjects. Branded (—■—); Generic A (---○---); Generic B (····●····)

Table 3

Values of plasma pharmacokinetic parameters obtained from 24 adult healthy volunteers following the administration of branded and two generic amoxicillin formulations

Formulations	AUC ($\text{h} \times \text{mg l}^{-1}$)	C_{max} (mg l^{-1})	T_{max} (h)
Branded	54.9 \pm 29.6 (46.3)*	16.1 \pm 8.8 (13.8)*	2.04 \pm 0.99 (2.00)†
Generic A	50.2 \pm 24.9 (43.6)*	14.1 \pm 6.8 (13.1)*	2.08 \pm 0.83 (2.00)†
Generic B	51.9 \pm 25.4 (45.0)*	15.7 \pm 8.9 (13.3)*	1.96 \pm 0.90 (2.00)†

Values are expressed as arithmetic mean \pm standard deviation, geometric mean (AUC and C_{max} values), and median (T_{max}). *Geometric mean. †Median. AUC, area under the time–concentration curve; C_{max} , maximal plasma concentration; T_{max} , time to achieve C_{max} .

5.4% greater than that estimated for generic A and B products, respectively, on the basis of arithmetic mean values.

Bioequivalence assessment

Prior to bioequivalence assessment, statistical analysis indicated the lack of period and sequence effects for both C_{max} and AUC. Indeed, the *P*-values of the period and sequence analysis were 0.763 and 0.467, respectively. For AUC, *P*-values were 0.563 and 0.756, respectively. Coefficients of variation for C_{max} and AUC were <30% (27.6 and 27.4%, respectively). When comparing generic formulations with branded amoxicillin, 90% CIs of AUC ratios were 0.8238, 1.0502 (ratio 0.9302) and 0.8116, 1.1007 (ratio 0.9452) for generic A and B vs. branded amoxicillin, respectively. In the same setting, 90% CIs of C_{max} ratios were 0.7921, 1.0134 (ratio 0.8960) and 0.8246, 1.1199 (ratio 0.9610) for generic A and B vs. branded amoxicillin, respectively. Upon comparison of generic A with generic B, the 90% CI of AUC ratio was 0.8400, 1.1528 (ratio 0.9841) and the 90% CI of C_{max} was 0.7972, 1.0863 (ratio 0.9307). Thus, on the basis of EMEA guidelines, generic A did not strictly satisfy the criteria for pharmacokinetic bioequivalence of C_{max} vs. branded amoxicillin. Moreover, lack of bioequivalence was found when comparing generic A with generic B also on C_{max} , the lower limit of the CI being in both cases just outside of the predefined range of 0.8, 1.25.

Discussion

The use of pharmacokinetic bioequivalence to demonstrate that generic and brand-name drugs are essentially similar in terms of efficacy and tolerability is currently a matter of discussion [15, 16]. Although the clinical development of a brand-name drug requires accurate characterization of its pharmacokinetics, efficacy and tolerability both in normal subjects and in the target patient population, in most cases the development of a generic drug

relies on the demonstration of its single-dose pharmacokinetic bioequivalence with the branded product in healthy volunteers [4]. In some instances, it is allowed to waive *in vivo* bioequivalence studies in favour of *in vitro* dissolution tests on oral immediate-release products with systemic actions, based on the criteria established by the Biopharmaceutics Classification System (BCS) [7]. However, this approach, which is restricted to noncritical drugs in terms of solubility, permeability and therapeutic range, such as amoxicillin [8], is still rarely used. Moreover, since guideline recommendations on biowaiving are fairly arbitrary and there is no harmonized assessment of BCS-based procedures within the European Community, biowaiver applications are generally rejected [9].

Pharmacokinetic bioequivalence studies employ healthy volunteers to minimize the magnitude of interindividual variability and are based on crossover designs to abate intraindividual variability. Besides generic drug registration, these studies are widely employed in other areas of clinical pharmacology, such as the development of new drugs, to compare different forms of the same active ingredient. In these cases, the pharmacokinetic and pharmacodynamic profiles of the new formulation are evaluated in conjunction with trials designed to prove efficacy and characterize tolerability [5]. However, when considering generic drugs, single-dose pharmacokinetics in healthy subjects might not accurately predict pharmacokinetic profiles in specific patient subpopulations, since it is recognized that drug pharmacokinetics in patients can be affected by a number of factors, including concomitant diseases, differences in first-pass metabolism, drug–drug interactions, diet and gastrointestinal conditions [4]. In this respect, some authors claim that single-dose bioequivalence studies in healthy volunteers might not reflect therapeutic equivalence in patients, particularly in the case of drugs characterized by a narrow therapeutic index or indicated for treatment of critical diseases, such as antiepileptics and antiarrhythmics [6, 17].

Based on these considerations, we performed the present postmarketing bioequivalence study to compare the branded amoxicillin product with two generic formulations, selected from the Italian market, to verify whether marketed generic formulations differ from their respective branded preparation. Of note, the selection of generic amoxicillin products was performed by means of an *in vitro* test that was simplified with respect to the standard method of US Pharmacopoeia [12], and therefore our assay might have over-discriminated the differences between generic formulations and brand leader amoxicillin. The pharmacokinetic study was conducted in both adult men and women, to approach the standard general population exposed to generic medicinal products. International guidelines suggest that bioequivalence investigations should be performed on a minimum of 12 subjects and the number of subjects must be calculated to ensure a power of at least 80%. However, 24 healthy volunteers were

enrolled in the present trial both to maintain adequate statistical power, as shown by post hoc analysis, and to overcome variability resulting from gender differences. In this respect, the sample size of this investigation was sufficient to minimize β -errors, and the randomization of study groups was sufficiently balanced to avoid bias of sequence allocation. Moreover, statistical analysis demonstrated that sequence and period effects did not occur. Overall, one of the two generic formulations (generic A) analysed in the present study did not satisfy the criteria for pharmacokinetic bioequivalence vs. branded amoxicillin, since the 90% CI interval of C_{\max} ratio just exceeded the lower limit. Indeed, bioequivalence can be claimed when the CI of both C_{\max} and AUC ratios falls within the range of 0.80, 1.25. It is also noteworthy that the AUC value, obtained for the branded formulation, was greater than that estimated for generic A and B, respectively.

The present observations open interesting perspectives for the discussion of the quality of generic drugs in the postmarketing setting. Our trial was conducted on a well-tolerated drug, such as amoxicillin, since it is unethical to test drugs with narrow therapeutic index in studies on healthy volunteers, and therefore the lack of bioequivalence might be a relevant issue, more in terms of pharmacokinetic/pharmacodynamic activity (i.e. antimicrobial efficacy) than safety. However, the efficacy of antibiotics is also closely related to the MIC of target bacteria [10], and therefore this parameter should be taken into account when the reference drug is an antibacterial agent [18]. When considering our findings on the lack of pharmacokinetic bioequivalence of generic A in light of the possible consequences for the pharmacodynamic activity of amoxicillin, it is of note that C_{\max} is not the major pharmacokinetic parameter to predict amoxicillin efficacy. Furthermore, the lower limit of 90% CI for C_{\max} of generic A vs. branded amoxicillin (0.7921) fell just below the acceptance limit of 0.80. Such a small gap might not be significant in the case of highly variable drugs, which are characterized by an intraindividual variability $>30\%$ [15], and it must be noted that EMEA guidelines allow widening of the acceptance range for 90% CI (0.75, 1.33) in some limited circumstances, including drugs with high within-subject variability [15]. In the present study, the intraindividual variability of amoxicillin formulations could not be assessed, since a replicate design is needed to obtain this information. However, our coefficients of variation, which can reflect several sources of variability, including within-subject variability, analytical errors and subject-by-formulation interaction, were $<30\%$ (i.e. 27.6% for C_{\max} and 27.4% for AUC), thus suggesting that a high level of within-subject variability was not likely to occur in this trial. As regards drugs with a narrow therapeutic index, the loss of interchangeability in the postmarketing phase might result in increased risk of adverse effects in target populations of patients exposed to the generic formulations, as pointed out by Crawford *et al.* [6] for antiepileptics and

Reiffel [17] for antiarrhythmics. For these reasons, international guidelines recommend specific procedures for products with a narrow therapeutic index, including the reduction of the 90% CI limits from 0.80, 1.25 to 0.90, 1.11 [1, 3].

Postmarketing evaluations highlighting lack of equivalence between generics and branded drugs have been previously reported in the medical literature. One case is represented by the study of Elkoshi *et al.* [19], who compared two formulations of omeprazole and observed that these products were not bioequivalent owing to differences in the composition of their enteric coating. Omeprazole, like other inhibitors of gastric proton pump, is employed on a short-term basis in combination with antibiotics (including amoxicillin) for the eradication of *Helicobacter pylori* [20], as well as to maintain long-term acid inhibition in patients with gastro-oesophageal reflux [21]. Since the absorption of omeprazole increases after repeated administrations, as a consequence of the decreased luminal acidity leading to reduced destruction of the active ingredient [22], it is conceivable that omeprazole formulations with altered performance of their enteric coating will display decreased bioavailability, and hence impaired control of acid secretion over time.

Chemical composition of medicinal products, variations in excipients, and the fact that brand leaders may have formulations and manufacturing processes that are about 20 years old at the time of generic development, are relevant issues to be considered as possible causes of non-equivalence among drug formulations. Interchangeability of generic and brand-name drugs does not necessarily imply that these products are identical in terms of chemical composition. The active ingredients must be the same, but their physicochemical properties can differ in several respects (e.g. conversion of a free base or acid into a salt). Furthermore, excipients and inactive ingredients may vary, and there is evidence in the literature to suggest that these changes can significantly affect the absorption kinetics and biological performance of drug formulations [23].

The possibility that various salts of the same active ingredient display distinct physicochemical and biological properties, which may result in differences in their clinical efficacy and safety, is of particular interest. For instance, when comparing the pharmacokinetics of penicillin free acid with three salted forms (sodium, potassium and calcium) of the same antibiotic, significant differences in both AUC and C_{\max} values have been observed [24]. In the present study, both the branded and generic amoxicillin formulations contained the same salt of the active ingredient, but differed for excipient composition, as shown in Table 1. However, whether such differences may have contributed to the lack of bioequivalence of generic A, as observed in our pharmacokinetic trial, remains undetermined, since specific data and detailed information on the manufacturing processes are not available. It is currently acknowledged that, if single-dose bioequivalence studies

in healthy subjects show similarity between formulations containing the active ingredient and excipients with different physical/chemical properties, these findings can be taken as evidence supporting equivalence in terms of therapeutic effectiveness and safety [5]. However, it remains undetermined whether, and to what extent, therapeutic equivalence can be maintained in patients receiving those formulations in repeated dosing regimens.

According to the results of our pharmacokinetic trial, generic A and B preparations can not be claimed as interchangeable on the basis of pharmacokinetic bioequivalence testing. This finding raises another relevant issue on generic drug prescription, since patients requiring long-term drug treatment are likely to receive over time generic copies of the same active ingredient manufactured by different companies. As a consequence, patients might be subjected to variations of the steady-state pharmacokinetic parameters, and hence possible therapeutic failures and/or adverse effects, which might result from the lack of bioequivalence between different generic products, with particular regard to drugs with narrow therapeutic index and/or indicated for critical pathological conditions.

In conclusion, one of the two amoxicillin generic products analysed in the present study is not equivalent to the brand leader formulation in terms of single-dose clinical pharmacokinetics. This finding supports the view that some generic drug products, granted as bioequivalent by the regulatory authorities, may lack actual interchangeability in the postmarketing setting, at least as regards the Italian market. Therefore, it is suggested that postmarketing bioequivalence studies on generic medicinal products should be performed with more advanced therapeutic equivalence methods such as steady-state pharmacokinetics and/or assessment of pharmacokinetic/pharmacodynamic relationships, in order to ensure adequate monitoring of the quality of generic drugs.

Competing interests

None declared.

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REFERENCES

- 1 The European Agency for the Evaluation of Medicinal Products (EMA). Note for guidance on the investigation of bioavailability and bioequivalence. 2001. CPMP/EWP/QWP/1401/98. Available at <http://www.emea.europa.eu/pdfs/human/ewp/140198en.pdf> (last accessed 18 December 2008).
- 2 Department of Health and Human Services Food and Drug Administration (HHS/FDA). Guidance for industry: bioavailability and bioequivalence studies for orally administered drug products – general considerations. 2003. Available at <http://www.fda.gov/cder/guidance/index.htm> (last accessed 18 December 2008).
- 3 World Health Organization (WHO). Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability. 2005. Working document QAS/04.093/Rev.4. Available at http://www.who.int/medicines/services/expertcommittees/pharmprep/QAS04_093Rev4_final.pdf (last accessed 18 December 2008).
- 4 Meredith P. Bioequivalence and other unresolved issues in generic drug substitution. *Clin Ther* 2003; 25: 2875–90.
- 5 Chen ML, Shah V, Patnaik R, Adams W, Hussain A, Conner D, Mehta M, Malinowski H, Lazor J, Huang SM, Hare D, Leske L, Sporn D, Williams R. Bioavailability and bioequivalence: an FDA regulatory overview. *Pharm Res* 2001; 18: 1645–50.
- 6 Crawford P, Feely M, Guberman A, Kramer G. Are there potential problems with generic substitution of antiepileptic drugs? A review of issues. *Seizure* 2006; 15: 165–76.
- 7 Yu LX, Amidon GL, Polli JE, Zhao H, Mehta MU, Conner DP, Shah VP, Lesko LJ, Chen ML, Lee VH, Hussain AS. Biopharmaceutics Classification System: the scientific basis for biowaiver extensions. *Pharm Res* 2002; 19: 921–5.
- 8 World Health Organization (WHO). Proposal to waive *in vivo* bioequivalence requirements for the WHO model list of essential medicines immediate release, solid oral dosage forms. 2005. Working document QAS/04.109/Rev.1. Available at http://www.who.int/medicines/services/expertcommittees/pharmprep/conf_materials/en/index.html (last accessed 18 December 2008).
- 9 The European Agency for the Evaluation of Medicinal Products (EMA). Concept paper on BCS-based biowaiver. 2007. EMEA/CHMP/EWP/213035/2007. Available at <http://www.emea.europa.eu/pdfs/human/ewp/21303507en.pdf> (last accessed 18 December 2008).
- 10 Scaglione F, Paraboni L. Influence of pharmacokinetics/pharmacodynamics of antibacterials in their dosing regimen selection. *Expert Rev Anti Infect Ther* 2006; 4: 479–90.
- 11 Leach AJ, Morris PS, Mathews JD; Chronic Otitis Media Intervention Trial – One (COMIT1) Group. Compared to placebo, long-term antibiotics resolve otitis media with effusion (OME) and prevent acute otitis media with perforation (AOMwIP) in a high-risk population: a randomized controlled trial. *BMC Pediatr* 2008; 8: 23–34.
- 12 USP, The United States Pharmacopeia, 24th edn, Rockville, MD: United States Pharmacopeial Convention Inc., 2000.
- 13 Petri WA. Penicillins, cephalosporins, and other beta-lactam antibiotics. In: *The Pharmacological Basis of Therapeutics*, 12th edn, ed. Bruton LL. New York, NY: McGraw-Hill, 2006; 1127–54.

- 14** Du X, Li C, Sun HK, Nightingale CH, Nicolau DP. A sensitive assay of amoxicillin in mouse serum and broncho-alveolar lavage fluid by liquid-liquid extraction and reversed-phase HPLC. *J Pharm Biomed Anal* 2005; 5: 648–52.
- 15** The European Agency for the Evaluation of Medicinal Products (EMA). Questions & answers on the bioavailability and bioequivalence guideline. 2007. EMA/CHMP/EWP/40326/2006. Available at <http://www.ema.europa.eu/pdfs/human/ewp/4032606en.pdf> (last accessed 18 December 2008).
- 16** The European Agency for the Evaluation of Medicinal Products (EMA). Recommendation on the need for revision of (CHMP) (Note for guidance on the investigation of bioavailability and bioequivalence). 2007. CPMP/EWP/QWP/1401/98. EMA/CHMP/EWP/200943/2007. Available at <http://www.ema.europa.eu/pdfs/human/ewp/20094307en.pdf> (last accessed 18 December 2008).
- 17** Reiffel JA. Issues in the use of generic antiarrhythmic drugs. *Curr Opin Cardiol* 2001; 16: 23–9.
- 18** Concia E, Novelli A, Schito GC, Marchese A. Ideal microbiological and pharmacological characteristics of a quality antimicrobial agent: comparing original and generic molecules. *J Chemother* 2007; 6: 609–19.
- 19** Elkoshi Z, Behr D, Mirimsky A. Multiple-dose studies can be a more sensitive assessment for bioequivalence than single-dose studies: the case with omeprazole. *Clin Drug Invest* 2002; 22: 585–92.
- 20** Murakami K, Okimoto T, Kodama M, Sato R, Watanabe K, Fujioka T. Evaluation of three different proton pump inhibitors with amoxicillin and metronidazole in retreatment for *Helicobacter pylori* infection. *J Clin Gastroenterol* 2008; 42: 139–42.
- 21** Edwards SJ, Lind T, Lundell L. Systematic review: proton pump inhibitors (PPIs) for the healing of reflux oesophagitis – a comparison of esomeprazole with other PPIs. *Aliment Pharmacol Ther* 2006; 24: 743–50.
- 22** Hassan-Alin M, Andersson T, Bredberg E, Röhss K. Pharmacokinetics of esomeprazole after oral and intravenous administration of single and repeated doses to healthy subjects. *Eur J Clin Pharmacol* 2000; 56: 665–70.
- 23** Verbeeck RK, Kanfer I, Walker RB. Generic substitution: the use of medicinal products containing different salts and implications for safety and efficacy. *Eur J Pharm Sci* 2006; 28: 1–6.
- 24** Davies G. Changing the salt, changing the drug. *Pharm J* 2001; 266: 322–3.